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(54) Title: CRYPTIC PEPTIDES AND METHOD FOR THEIR IDENTIFICATION

(57) Abstract

The invention provides a method of determining whether a peptide of a protein is a cryptic peptide, which method includes the steps of: i) exposing T cells with the peptide in a primary challenge; ii) measuring the reactivity of T cells with the peptide in the primary challenge of step i); iii) exposing pre-challenged T cells with the peptide in a secondary challenge, wherein the pre-challenged T cells are obtainable by exposing the T cells to the protein; and measuring the reactivity of the pre-challenged T cells with the peptide in the secondary challenge of step iii), and the peptide is a cryptic peptide if T-cell reactivity is observable in the secondary challenge but not in the primary challenge.

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CRYPTIC PEPTIDES AND METHOD FOR THEIR IDENTIFICATION

The present invention relates to immunologically cryptic peptides; methods for their identification in individuals and populations and their use in diagnosis and therapy of pathological conditions such as asthma and allergy, and their use in screening for therapeutic activity.

The ability of the immune system to elicit a response to a particular molecule depends critically upon its ability to recognise the presence of an antigen. Classically, the term antigen was associated with the ability of a molecule to be an antibody generator *via* induction of B-cells. It is now known however that T cells also possess the ability to recognise antigens. T-cell antigen recognition requires antigen presenting cells (APCs) to present antigen fragments (peptides) on their cell surface in association with molecules of the major histocompatibility complex (MHC). T cells use their antigen specific receptors (TCRs) to recognise the antigen fragments presented by the APC. Such recognition acts as a trigger to the immune system to generate a range of responses to eradicate the antigen which has been recognised.

In order to trigger an individual T cell, a critical number of TCRs must be ligated by the peptide/MHC complex presented by the APC. A peptide which reaches the surface of the APC in sufficient numbers to do this can be termed "dominant" or "sub-dominant" depending upon its ability to induce T cell activation relative to other triggering peptides. In the normal course of events i.e. physiologically, a given protein will generate more than one peptide which is capable of triggering a T cell response. The term "dominant" would then be applied to the peptide that induces the most potent or most frequent response. In addition to dominant and sub-dominant epitopes, there are potential T cell peptide epitopes within a given protein sequence (for which T cells are specific), which do not reach the APC surface in sufficient numbers to induce a response. In other words due to the mechanisms of antigen processing within the APC, certain peptides are processed and presented to T-lymphocytes efficiently and therefore stimulate T-cell responses while others are poorly processed and presented to T-lymphocytes. As these latter peptides are not present upon the APC surface in sufficient numbers to

stimulate a potentially reactive T-lymphocyte. these peptides have been referred to as "cryptic peptide epitopes".

Cryptic peptide epitopes are present both in proteins normally present in the body (self proteins) and in non-self (or foreign) proteins. In normal physiology, T cells which have the capability of reacting with a cryptic epitope cannot be detected in an *in vitro* primary stimulation assay (that is, T cells freshly isolated from the blood do not exhibit demonstrable proliferation when cultured with the cryptic peptide). In contrast other peptides which are efficiently processed and presented to T-lymphocytes by APC, can stimulate a proliferative response in primary culture. These are the "dominant" and "sub-dominant" epitopes.

The term "atopic allergy" is applied to a group of allergies characterised by high concentrations of immunoglobulin E (IgE). They include allergic asthma, hay fever, perennial allergic rhinitis, some forms of urticaria (hives) and eczema, allergic conjunctivitis and certain food allergies (particularly food anaphylaxis). The mechanisms of generation of the pathology of such atopic conditions involves not only the synthesis of antigen/allergen specific IgE but also the accompanying differentiation and growth of effector cells such as mast cells and eosinophils.

Allergic IgE-mediated diseases are currently treated by desensitization procedures that involve the periodic injection of allergen components or extracts. Desensitization treatments may induce an IgG response that competes with IgE for allergen, or they may induce specific suppressor T cells that block the synthesis of IgE directed against allergen. This form of treatment is not always effective and poses the risk of provoking serious side effects, particularly general anaphylactic shock. This can be fatal unless recognised immediately and treated with adrenaline. A therapeutic treatment that would decrease or eliminate the unwanted allergic-immune response to a particular allergen, without altering the immune reactivity to other foreign antigens or triggering an allergic response itself would be of great benefit to allergic individuals.

Sometimes the normal mechanisms whereby self and non-self are immunologically distinguished may break down and an immune response may be elicited against self-antigens present in normal body tissues. This "auto-immunity" generates pathological

conditions such as autoimmune thyroiditis, rheumatoid arthritis and lupus erythematosus. Therapeutic regimes are generally limited to the use of anti-inflammatory or immunosuppressive drugs which are relatively non-specific and have many undesirable side-effects.

WO 92/11859 describes a method of reducing immune response to an allergen in which a non-allergen derived, non-stimulating peptide which binds to specific MHC class II molecules of APCs is used to inhibit T-cell response to particular allergens.

WO 91/06571 purports to disclose peptides derived from human T-cell reactive feline protein which can be used in the diagnosis, treatment or prevention of cat allergy.

WO 94/24281 relates to peptides and modified peptides of the major house dust mite allergens. The modified peptides have the intent of reducing the level of undesirable side effects associated with desensitizing therapies.

G. F. Hoyne et. al. in Immunology 83 pp 190-195 (1994) examined house dust mite allergy using peptides made from cDNA encoding the major allergen DerpI. They purport to show that peptides containing major epitopes can induce oral tolerance in mice to the whole allergen and that it is also possible to induce tolerance with other peptides. Cryptic peptides are suggested as playing a role in this process but no methods are disclosed for their identification or therapeutic use.

None of the above disclosures makes any suggestion that cryptic peptides may play a role in the pathology of atopic conditions such as asthma or other allergic diseases. The present inventors have found a method for identifying cryptic peptides and have observed that individuals with asthma or other allergy-based pathologies have T-lymphocyte populations which can be stimulated in primary culture by cryptic epitopes derived from the allergen which causes the relevant pathology. As described above, T-lymphocytes isolated from a healthy individual would not be expected to be stimulated in primary culture by a cryptic epitope. Likewise in autoimmune pathologies, a self peptide, normally cryptic to the immune system becomes recognised and elicits an immune response.

Hence there is provided according to the invention, a method of determining whether a peptide of a protein is a cryptic peptide, which method includes the steps of:

i) exposing T cells with the peptide in a primary challenge; ii) measuring the reactivity of T cells with the peptide in the primary challenge of Step i; iii) exposing pre-challenged T cells with the peptide in a secondary challenge, wherein the pre-challenged T cells are obtainable by exposing the T cells to the protein; and measuring the reactivity of the pre-challenged T cells with the peptide in the secondary challenge.

The prechallenge allows expression of not only dominant and sub-dominant epitopes on the APC surface, but also of any cryptic determinants. The subsequent peptide rechallenge of these cells reveals T-cell reactivity to the dominant, sub-dominant and cryptic epitopes. Primary challenge with peptides will elicit responses from only the normally expressed dominant and sub-dominant epitopes. Thus, peptides recognised following whole antigen primary challenge followed by peptide secondary challenge, but not after peptide primary challenge alone are by definition, cryptic epitopes. The peptide is a cryptic peptide if T-cell reactivity is observable in the secondary challenge above but not in the primary challenge.

The present invention may be illustrated as follows. If peripheral blood mononuclear cells (PBMC) which contain T cells are taken directly from an individual and incubated for a short period of time (e.g. 3-7 days) with a set of synthetic, overlapping peptides from a protein, proliferative responses will be seen to the peptides which T cells recognise in the normal course of events i.e. dominant and sub-dominant epitopes. If, however, PBMC are first cultured for 1-2 weeks with a high dose of the whole molecule (or a cocktail of all the peptides), potential T-cell epitopes dominant, sub-dominant and cryptic will then be detected by subsequent "secondary" challenge with the peptides. This is because T-cell populations with any specificity for any of the peptides will have been triggered and expanded (increased in numbers) as a result of the high dose "primary" challenge with whole antigen. By challenging PBMC with the peptides in a primary assay and also a secondary assay, cryptic epitopes can be identified within a population. Therefore any peptide which behaves as a dominant epitope in asthmatics or individuals with a related pathology, but as a cryptic epitope in the normal population can be detected using this method of primary and secondary assay.

In preferred embodiments of the present invention the pre-challenged cells are obtained by exposing the T cells to protein or by exposing the T cells to protein in bulk culture.

The T cells may be obtained from a population comprising a number of individuals (e.g. > 20) or from a single individual. If they are obtained from a population then any peptide identified as cryptic in all healthy individuals may be considered to be cryptic within the population in general while use of T cells isolated from a single individual will identify only peptides which are cryptic (or not) in that individual and may not be cryptic in the population.

The steps of the method above may be carried out in the sequence as described or in any alternative sequence known to the person skilled in the art to be suitable to obtain an essentially equivalent result.

In a particularly preferred embodiment of the present invention, the protein from which the peptides are derived is chosen from the list comprising Fel dI (the feline skin and salivary gland allergen of the domestic cat Felis domesticus - the amino acid sequence of which is disclosed in WO 91/06571), Der p I, Der p II, Der fI or Der fII (the major protein allergens from the house dust mite dermatophagoides - amino acid sequences disclosed in WO 94/24281) and allergens present in any of the following: grass, tree and weed (including ragweed) pollens; fungi and molds; foods e.g. fish, shellfish, crab, lobster, peanuts, nuts, egg and milk; stinging insects e.g. bee, wasp and hornet and the chironomidae (non-biting midges); spiders and mites; mammals such as dog, horse, rat, guinea pig, mice and gerbil; latex; biological detergent additives; drugs e.g. penicillins and other antibiotics and anaesthetic agents.

More particularly the insect protein from which the peptides may be derived is chosen from the list comprising: housefly, fruit fly, sheep blow fly, screw worm fly, grain weevel, silkworm, honeybee, non-biting midge larvae, bee moth larvae, mealworm, cockroach and larvae of Tenibrio molitor beetle. All these being insect allergens of particular relevance to allergic problems arising in the workplace.

Particularly preferred peptides for use in the method of the invention include those shown in Figs. 1, 2 and 3. The method may also use those shown in any one of

Sequence I.D. Nos 25 to 56. The invention also encompasses the use of these peptides as cryptic peptides.

A further aspect of the present invention is any peptide identified as being cryptic when screened by the method of the present invention.

Peptides retaining the ability to bind to MHC class II molecules may have up to around 40, preferably 31 residues. Thus, it will be appreciated that useful peptides may comprise a sequence as shown in any one of Sequence I.D. Nos 2 to 10 and 12 to 56. Thus, in one preferred example a 14mer contiguous sequence forms part of a larger peptide, preferably one up to about 31 residues. In this example the 14mer forms about 45% of the larger peptide (or polypeptide). Preferably the sequence forms about 50% or more, more preferably about 60% or more, even more preferably about 70% or more, or about 80% or more of the larger peptide. In a specially preferred embodiment the sequence forms about 90% or about 95% or more of the larger sequence.

Yet further aspects of the present invention include a peptide of the present invention, preferably a cryptic peptide when screened by the method of the present invention for use as a medicament or as a diagnostic; the use of a peptide of the present invention, preferably a cryptic peptide when screened by the method of the present invention, in the preparation of a medicament for the treatment of atopic conditions such as asthma; the use of a peptide of the present invention, preferably a cryptic peptide when screened by the method of the present invention, in the preparation of a diagnostic for the diagnosis of atopic conditions such as asthma; a method of preparing a medicament or diagnostic comprising mixing a peptide of the present invention, preferably a cryptic peptide when screened by the method of the present invention, with a suitable carrier, diluent or excipient; the formulations prepared from such uses and methods; a method of therapy and/or diagnosis practised on the human body using a peptide of the present invention, preferably a cryptic peptide.

Whilst it may be possible for the peptides of the present invention to be administered as the raw peptide, it is preferable to present them as a pharmaceutical formulation. According to a further aspect, the present invention provides a pharmaceutical formulation comprising a cryptic peptide together with one or more

pharmaceutically acceptable carriers therefor and optionally one or more other therapeutic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for oral (particularly inhaled), parenteral (including subcutaneous, transdermal, intradermal, intramuscular and intravenous and rectal) administration although the most suitable route may depend upon for example the condition and disorder of the recipient. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association a compound of the present invention as herein defined or a pharmacologically acceptable salt or solvate thereof ("active ingredient") with the carrier which constitutes one or more accessory ingredients.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Formulations for inhalation may be presented in any of the ways known to be effective e.g. metered does inhalers.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example, water-for-injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter or polyethylene glycol.

Preferred unit dosage formulations are those containing an effective dose, as hereinbelow recited, or an appropriate fraction thereof, of the active ingredient.

The compounds of the invention may typically be administered orally or via injection at a dose of from 0.001 to 1 mg/kg per day.

The present invention also provides diagnostic kits which comprise cryptic peptides of the present invention. Each kit consists of a microculture plate containing lyophilized peptides corresponding to the cryptic peptide(s) identified by the method of the invention. Each peptide may be present in a minimum of three wells (for statistical evaluation). More replicates may be used depending on the number of peptides to be screened. The use of lyophilized peptides allows the kit to be stored at room temperature for several months before use.

According to a preferred embodiment for carrying out the assay, peripheral blood mononuclear cells (PBMC) may be isolated from patient blood by standard methods. Approximately 20 ml of blood is required. PBMC is added to the wells of the microculture plate in a volume of 200μl of culture medium containing 10^5 cells. After 6 days of culture in a humidified incubator at 37°C gased with 5% CO_2 in air, an isotopic label such as ^{3}H-thymidine or, a non-isotopic label such as bromo-deoxyuridine is added to each well for a prescribed period (approx. 12-24 hours). Cell proliferation to the peptides present may be assayed in any suitable way, e.g. DNA synthetic analysis, liquid scintillation spectroscopy or colourimetrically.

The measurement of cellular reactivity to peptide challenge according to the present invention is preferably carried out by measurement of cellular proliferation as discussed above. However such reactivity may also be measured by determination of other cellular responses e.g. assay for the release of soluble mediators such as cytokines and chemokines which may demonstrate a release profile indicative of allergic responses.

Cryptic peptides of the present invention which are to be used in therapy may be subject to point mutation in order to avoid certain undesirable side effect of peptide immunotherapy and/or to improve their effects.

Peptide immunotherapy involves the administration of peptides which will disable specific T cells. Data from the trial of Allervax Cat [J. Allergy Clin. Immunol. Jan 1996 Abstract 815] shows that in some patients, a late-phase response to the administered peptide occurs. This response is essentially an asthma attack and represents a serious side-effect of this kind of therapy. By use of "altered peptide ligands" the same therapeutic goals can be achieved i.e., functional elimination of reactive T cells, but without the development of a late-phase response. Having identified a cryptic peptide for therapeutic use, a series of a point mutations may be made to residues in order to generate a panel of closely related peptides. These may be screened for there ability to a) bind to the appropriate MHC molecule with a similar affinity to the original peptide and b) anergise or kill T cells specific for the original peptide. Such altered or synthetic peptides are also encompassed within the present invention.

Particularly preferred synthetic peptides, according to the present invention and for use according to the present invention, include those shown in Figure 3. Such peptides may be used according to the present invention either individually or in any pairwise or multiple combination thereof.

The pre-challenged T cells may be generated as described above or in one alternative they may be generated by exposure of the T cells to bulk culture or to multiple peptides derived from the protein of interest.

In a particularly preferred embodiment according to the present invention, the method for determining whether a peptide of a protein is a cryptic peptide may be carried out as follows. T cells are isolated from peripheral blood using established techniques [Cellular Immunology LabFax., P.J. Delves (Ed), 1994, Tissue and Cell Culture, Chapter 4, pp. 45. Blackwell Scientific Publications]. A proportion of the cells are cultured *in vitro* for 6-12 days with the protein of interest. The rest of the cells are aliquoted into cultures and incubated with the peptide of interest derived from the protein. After 3-6 days a label is added to the culture which enables the quantity of DNA synthesis within the cultures to be quantified sometime later (e.g. 8-16 hours later). By analysing the quantity of DNA in the cultures compared to a control culture containing no peptide, it is possible to identify peptide sequences from the protein which stimulate

T cells in this "primary" challenge. The group of cells cultured for 6-12 days with whole protein are collected at the end of this culture and washed several times in culture medium. However, as these cells were cultured with whole protein first i.e. were "pre-challenged" the subsequent peptide challenges are referred to as "secondary" challenges. Subsequent analysis of quantities of DNA synthesis in secondary challenges will identify all peptides derived from the protein which can potentially be recognised by T cells within an individual.

In summary, the primary cultures identify the peptides which are recognised by T-lymphocytes from individuals with disease or without disease and the secondary cultures identify all peptides to which T cells can react in individuals with disease or without disease. Epitopes which are cryptic at the population level will be recognised in primary culture by individuals with disease but not by individuals without disease.

T cells according to the present invention may be taken to be any preparation of mononuclear cells obtained from one or more individuals containing T-lymphocytes at a purity sufficient to be able to detect reactivity in a peptide challenge. All technical processes described above may alternatively be carried out using another process known to the person skilled in the art to be able to achieve the desired purpose of that process.

In a further embodiment of the present invention, there is provided the use of cryptic peptides in a compound screen for the identification of compounds possessing therapeutic activity particularly in respect of atopic conditions and more particulary in respect of asthma. In this further embodiment of the present invention the cryptic peptides are preferably those identified using the method of the present invention.

According to this further embodiment of the present invention there is provided both a screening process for the identification of therapeutic compounds and a kit adapted to put such screening process into effect. The screening process of the present invention is preferably carried out *in vitro*, but may also be carried out *in vivo*. In a preferred embodiment, the screening process of the present invention may be carried out *in vitro* as follows. Isolated peripheral blood mononuclear cells or cultured T lymphocytes are cultured *in vitro* with concentrations of one or more cryptic peptides, preferably previously determined as being optimal for the induction of proliferative and/or other

responses in these cells. To certain of the culture wells are added, compounds which are to be screened for their potential to diminish the proliferative response of these cells to the cryptic peptide. Such compounds are preferably added to cultures over a broad range of concentrations. For example, the compound may be added to the cultures at 10-fold dilutions over the range 10^{-12}M to 10^{-2}M in order to identify the dose providing the largest diminution in the proliferative response of the cells. Cellular proliferation may be measured using any technique well known in the art, for example cells may be labelled with a compound such as tritiated thymidine which will enable quantification of DNA synthesis and thus cellular proliferation. Compounds capable of diminishing the cellular proliferation induced by the cryptic peptide may be identified as a therapeutic in respect of a medical condition associated with that cryptic peptide. When carried out *in vivo*, the screening process comprises the administration preferably to a human mammal, of a cryptic peptide to induce a condition associated with that cryptic peptide and the administration, preferably subsequently, of a compound to be screened. A compound capable of diminishing symptoms associated with the induced condition may be identified as a therapeutic in respect of that condition.

Preferably the screen whether *in vitro* or *in vivo* comprises the use of cryptic peptides associated with atopic conditions such as asthma and most preferably the use of one or more of the FC1P peptides identified herein.

Non-limiting examples of the invention will now be described with reference to the accompanying Figures, in which:-

Figure 1: shows the native sequence of chain 1 of the major cat allergen Fel d I in single letter amino acid code; and nine peptides derived therefrom.

Figure 2: shows the native sequence of chain 2 of the major cat allergen Fel d I in single letter amino acid code; and eleven peptides derived therefrom.

Figure 3: shows three synthetic peptides derived from the sequence of Fel d I. The three peptides are collectively referred to as FC1P.

Figure 4: shows the results of Example 2 in terms of levels of cellular proliferation (expressed as Δ cpm) in response to primary and secondary challenge of PBMCs with FC1P, as described of PBMCs in the Example.

Figure 5: shows the results of Example 3 in terms of levels of cellular proliferation (expressed as Δ cpm) in response to primary challenge of PBMCs with FC1P, as described in the Example.

Figure 6: shows the results of Example 4 in terms of the lung function of an asthmatic (expressed as FEV_1 in litres) over time, as a consequence of the factors described in the Example.

Figure 7: shows the results of Example 5 in terms of inhibition of T cell proliferation (measured by tritiated thymidine incorporation) to FC1 P peptides by dexamethazone and fluticazone.

EXAMPLE 1

The method for determining whether a peptide of a protein is a cryptic peptide may be carried out as follows.

1 - T cells are isolated from peripheral blood,

2 - a proportion of the cells are cultured *in vitro* for 6-12 days with the protein of interest.

3 - the rest of the cells are aliquoted into cultures and incubated with the peptide of interest derived from the protein,

4 - after 3-6 days a label is added to the culture, enabling the quantity of DNA synthesis within the cultures to be quantified.

5 - analysis of the quantity of DNA synthesis in the cultures compared to a control culture containing no peptide, this identifies the peptide sequences from the protein which stimulate T cells in this "primary" challenge,

6 - the group of cells cultured for 6-12 days with whole protein are collected at the end of this culture and washed several times in culture medium,

7 - analysis of quantities of DNA synthesis in secondary challenges will identify all peptides derived from the protein which can be recognised by T cells within an individual.

The cryptic peptides identified by this method are those stimulating T-cell reactivity in the secondary challenge but not in the primary challenge.

EXAMPLE 2

Peripheral blood mononuclear cells were isolated by density gradient centrifugation. A proportion of the cells were challenged with peptides in primary culture and the remaining cells cultured for 10 days with 100μg/ml cat dander extract (containing Fel d I). Subsequently, cells cultured with cat dander were collected, washed in culture medium and then cultured for a further three days with the three peptides (at a concentration of 36μg/ml) which together constitute FC1P (as shown in the Figure 3 herein) and equal numbers of irradiated autologous feeder cells. Cellular proliferation (expressed as Δ cpm) was quantified by measurement of incorporation of tritiated thymidine into newly synthesised DNA, and is illustrated in Figure 4.

EXAMPLE 3

Freshly isolated peripheral blood mononuclear cells were cultured at 10^5 cells per well for 6 days prior to labelling with tritiated thymidine for measurement of cellular proliferation. Cat allergic asthmatics (CAA) demonstrate greater primary proliferative responses to FC1P (36μm/ml) than do non-cat allergic asthmatics (NCA). The results are shown in Figure 5.

EXAMPLE 4

40μg of highly purified FC1P peptides was injected intradermally into each forearm of a volunteer cat-allergic asthmatic. This induced an isolated late-phase asthmatic reaction approximately three hours after administration of FC1P. Lung function

(quantified as FEV_1) fell and remained low over the next several hours. The drop in FEV_1 was reversed by the administration of $ß_2$ agonist and inhaled corticosteroid at 8 hours. The results of this are shown diagrammatically in Figure 6.

EXAMPLE 5

Freshly isolated T lymphocytes were cultured *in vitro* with concentrations of FC1P peptides, previously determined as being optimal for the induction of proliferative and/or other responses in these cells. To certain of the culture wells were added, the glucocorticosteroid Dexamethazone or fluticazone, these were added to cultures at dilutions of 10^{-6}M or 10^{-9}M. After culture, cells were labelled with tritiated thymidine to enable quantification of DNA synthesis and thus cellular proliferation. The results are shown in Figure 7.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: IMPERIAL COLLEGE OF SCIENCE TECHNOLOGY AND MEDICINE
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(E) COUNTRY: UNITED KINGDOM
(F) POSTAL CODE (ZIP): SW7 2AZ

(ii) TITLE OF INVENTION: PEPTIDES

(iii) NUMBER OF SEQUENCES: 56

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Glu Ile Cys Pro Ala Val Lys Arg Asp Val Asp Leu Phe Leu Thr Gly
1               5                  10                  15

Thr Pro Asp Glu Tyr Val Glu Gln Val Ala Gln Tyr Lys Ala Leu Pro
            20                  25                  30

Val Val Leu Glu Asn Ala Arg Ile Leu Lys Asn Cys Val Asp Ala Lys
        35                  40                  45

Met Thr Glu Glu Asp Lys Glu Asn Ala Leu Ser Leu Leu Asp Lys Ile
    50                  55                  60

Tyr Thr Ser Pro Leu Cys
65                  70
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Glu Ile Cys Pro Ala Val Lys Arg Asp Val Asp Leu Phe Leu Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Arg Asp Val Asp Leu Phe Leu Thr Gly Thr Pro Asp Glu Tyr Val Glu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Gly Thr Pro Asp Glu Tyr Val Glu Gln Val Ala Gln Tyr Lys Ala Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Glu Gln Val Ala Gln Tyr Lys Ala Leu Pro Val Val Leu Glu Asn Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Tyr Lys Ala Leu Pro Val Val Leu Glu Asn Ala Arg Ile Leu Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Pro Val Val Leu Glu Asn Ala Arg Ile Leu Lys Asn Cys Val Asp Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Arg Ile Leu Lys Asn Cys Val Asp Ala Lys Met Thr Glu Glu Asp Lys
1 5 10 15

Glu

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Lys Met Thr Glu Glu Asp Lys Glu Asn Ala Leu Ser Leu Leu Asp Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Asn Ala Leu Ser Leu Leu Asp Lys Ile Tyr Thr Ser Pro Leu Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 92 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Val Lys Met Ala Glu Thr Cys Pro Ile Phe Tyr Asp Val Phe Phe Ala
1 5 10 15

Val Ala Asn Gly Asn Glu Leu Leu Leu Lys Leu Ser Leu Thr Lys Val
20 25 30

Asn Ala Thr Glu Pro Glu Arg Thr Ala Met Lys Lys Ile Gln Asp Cys
35 40 45

Tyr Val Glu Asn Gly Leu Ile Ser Arg Val Leu Asp Gly Leu Val Met
50 55 60

Thr Thr Ile Ser Ser Ser Lys Asp Cys Met Gly Glu Ala Val Gln Asn
65 70 75 80

Thr Val Glu Asp Leu Lys Leu Asn Thr Leu Gly Arg
85 90

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Val Lys Met Ala Glu Thr Cys Pro Ile Phe Tyr Asp Val Phe Phe Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Ile Phe Tyr Asp Val Phe Phe Ala Val Ala Asn Gly Asn Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Val Ala Asn Gly Asn Glu Leu Leu Leu Lys Leu Ser Leu Thr Lys Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Glu Leu Leu Leu Lys Leu Ser Leu Thr Lys Val Asn Ala Thr Glu Pro
1 5 10 15

Glu

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Asn Ala Thr Glu Pro Glu Arg Thr Ala Met Lys Lys Ile Gln Asp
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Arg Thr Ala Met Lys Lys Ile Gln Asp Cys Tyr Val Glu Asn Gly Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Cys Tyr Val Glu Asn Gly Leu Ile Ser Arg Val Leu Asp Gly Leu Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Ile Ser Arg Val Leu Asp Gly Leu Val Met Thr Thr Ile Ser Ser Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Met Thr Thr Ile Ser Ser Ser Lys Asp Cys Met Gly Glu Ala Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Lys Asp Cys Met Gly Glu Ala Val Gln Asn Thr Val Glu Asp Leu Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Gln Asn Thr Val Glu Asp Leu Lys Leu Asn Thr Leu Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Leu Phe Leu Thr Gly Thr Pro Asp Glu Tyr Val Glu Gln Val Ala Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Lys Ala Leu Pro Val Val Leu Glu Asn Ala Arg Ile Leu Lys Asn Cys
1 5 10 15

Val

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Glu Ile Cys Pro Ala Val Lys Arg Asp Val Asp Leu Phe Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26 :

Ile Cys Pro Ala Val Lys Arg Asp Val Asp Leu Phe Leu Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Cys Pro Ala Val Lys Arg Asp Val Asp Leu Phe Leu Thr Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Pro Ala Val Lys Arg Asp Val Asp Leu Phe Leu Thr Gly Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Ala Val Lys Arg Asp Val Asp Leu Phe Leu Thr Gly Thr Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Val Lys Arg Asp Val Asp Leu Phe Leu Thr Gly Thr Pro Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Lys Arg Asp Val Asp Leu Phe Leu Thr Gly Thr Pro Asp Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Arg Asp Val Asp Leu Phe Leu Thr Gly Thr Pro Asp Glu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Asp Val Asp Leu Phe Leu Thr Gly Thr Pro Asp Glu Tyr Val
1 5 10

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Val Asp Leu Phe Leu Thr Gly Thr Pro Asp Glu Tyr Val Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

Asp Leu Phe Leu Thr Gly Thr Pro Asp Glu Tyr Val Glu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Leu Phe Leu Thr Gly Thr Pro Asp Glu Tyr Val Glu Gln Val
1 5 10

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

Phe Leu Thr Gly Thr Pro Asp Glu Tyr Val Glu Gln Val Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Leu Thr Gly Thr Pro Asp Glu Tyr Val Glu Gln Val Ala Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

Thr Gly Thr Pro Asp Glu Tyr Val Glu Gln Val Ala Gln Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

Gly Thr Pro Asp Glu Tyr Val Glu Gln Val Ala Gln Tyr Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

Thr Pro Asp Glu Tyr Val Glu Gln Val Ala Gln Tyr Lys Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

Pro Asp Glu Tyr Val Glu Gln Val Ala Gln Tyr Lys Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

Asp Glu Tyr Val Glu Gln Val Ala Gln Tyr Lys Ala Leu Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

Glu Tyr Val Glu Gln Val Ala Gln Tyr Lys Ala Leu Pro Val
1 5 10

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

Tyr Val Glu Gln Val Ala Gln Tyr Lys Ala Leu Pro Val Val
1 5 10

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

Val Glu Gln Val Ala Gln Tyr Lys Ala Leu Pro Val Val Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

Glu Gln Val Ala Gln Tyr Lys Ala Leu Pro Val Val Leu Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

Gln Val Ala Gln Tyr Lys Ala Leu Pro Val Val Leu Glu Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

Val Ala Gln Tyr Lys Ala Leu Pro Val Val Leu Glu Asn Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

Ala Gln Tyr Lys Ala Leu Pro Val Val Leu Glu Asn Ala Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

Gln Tyr Lys Ala Leu Pro Val Val Leu Glu Asn Ala Arg Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

Tyr Lys Ala Leu Pro Val Val Leu Glu Asn Ala Arg Ile Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

Lys Ala Leu Pro Val Val Leu Glu Asn Ala Arg Ile Leu Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

Ala Leu Pro Val Val Leu Glu Asn Ala Arg Ile Leu Lys Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

Leu Pro Val Val Leu Glu Asn Ala Arg Ile Leu Lys Asn Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

Pro Val Val Leu Glu Asn Ala Arg Ile Leu Lys Asn Cys Val
1 5 10

CLAIMS

1. A method of determining whether a peptide of a protein is a cryptic peptide, which method includes the steps of:

i. exposing T cells with the peptide in a primary challenge;

ii. measuring the reactivity of T cells with the peptide in the primary challenge of Step i;

iii. exposing pre-challenged T cells with the peptide in a secondary challenge, wherein the pre-challenged T cells are obtainable by exposing the T cells to the protein; and

iv. measuring the reactivity of the pre-challenged T cells with the peptide in the secondary challenge of Step iii;

wherein the peptide is a cryptic peptide if T cell reactivity is observable in the secondary challenge but not in the primary challenge.

2. A method according to claim 1 wherein Steps i to iv are carried out ex vivo.

3. A method according to claim 1 or claim 2 wherein the pre-challenged cells are obtained by exposing the T cells to the protein.

4. A method according to any one of claims 1 to 3 wherein the pre-challenged T cells in Step iii are prepared by exposing the T cells to the protein in a bulk culture.

5. A method according to any one of claims wherein Steps i to iv are not sequential.

6. A method according to any one of claims 1 to 5 wherein the protein is any one of Fel dI, Der pI, Der pII, Der fI and Der fII.

7. A method according to any one of Claims 1 to 5 wherein the protein is an allergenic protein derived from any one of grass, tree and weed pollens; fungi and molds, foods; insects; the chironomidae; spiders and mites; mammals, latex, biological detergent additives and drugs.

8. A method according to Claim 7 wherein the weed is ragweed; the mammal is a dog, cat, horse, rat, guinea pig, mouse or gerbil; the drug is an antibiotic or anaesthetic; and the insect is bee, wasp, hornet, housefly, fruit fly, sheep blow fly, screw worm fly, grain weevel, silkworm, honeybee, non-biting midge larvae, bee moth larvae, mealworm, cockroach or Tenibrio moliter beetle larvae.

9. A cryptic peptide when screened by the method according to any one of claims 1 to 8.

10. A method of altering a cryptic peptide according to claim 9 wherein point mutations are introduced using chemical or recombinant DNA techniques.

11. A cryptic peptide obtained by the method of claim 10.

12. A peptide as described in any one of Sequence I.D. Nos 1 to 56 herein.

13. A peptide of which about 45% or more is comprised of the sequence of any one of Sequence I.D. Nos 2 to 10 and 12 to 56.

14. A peptide according to any one of claims 9, and 11 to 13 for use as a medicament or as a diagnostic.

15. A method of preparing a medicament or a diagnostic, which method comprises mixing a peptide according to any one of claims 9, and 11 to 13, with a suitable carrier, diluent or excipient.

16. Use of a peptide according to any one of claims 9, and 11 to 13 in the manufacture of a medicament for the treatment of an atopic condition.

17. Use according to claim 16 wherein the atopic condition is asthma.

18. Use of a peptide according to any one of claims 9, and 11 to 13 in the manufacture of a diagnostic for the diagnosis of an atopic condition.

19. Use according to claim 18 wherein the atopic condition is asthma.

20. A process for screening a compound for therapeutic activity comprising measuring the ability of the compound to diminish cellular proliferation induced in a cell culture by a cryptic peptide.

21. A process according to claim 20 wherein the cell culture comprises peripheral blood mononuclear cells or T lymphocytes.

22. A process according to either of claims 20 and 21 wherein the peptide is one or more FC1P peptide.

23. A process according to any one of claims 20 to 21 wherein the cryptic peptide is as claimed in any one of claims 9, and 11 to 13.

24. A process according to any one of claims 20 to 23 wherein the screen is for asthma therapeutic activity.

25. A compound identified as possessing therapeutic activity by the process of any one of claims 20 to 24.

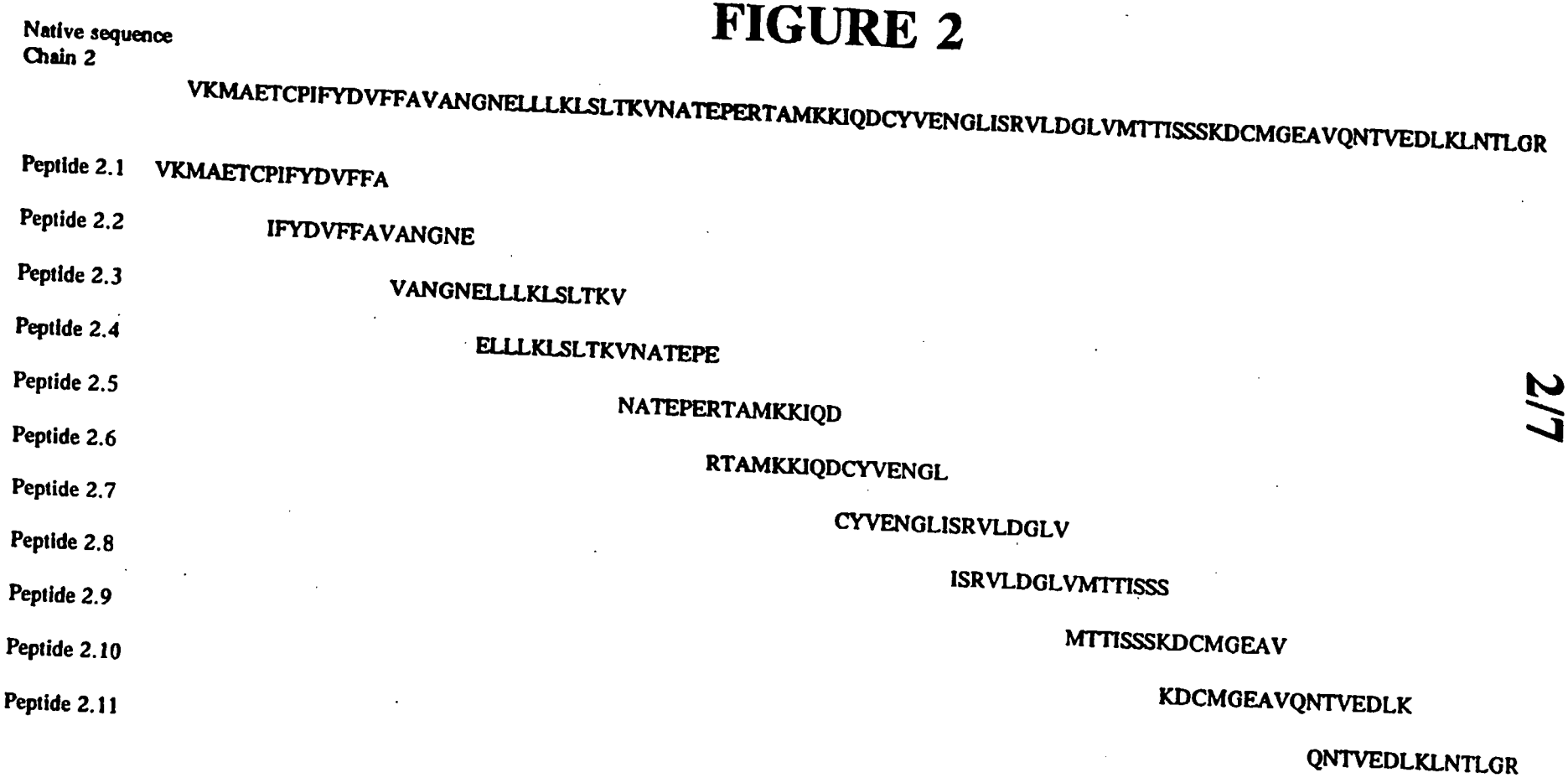
26. A kit comprising a cryptic peptide, adapted for use in the method of any one of claims 1 to 8 or the process of any one of claims 20 to 24.

27. A kit adapted for carrying out the method of any one of claims 1 to 8 or the process of any one of claims 20 to 24 comprising a microtitre plate and means for measuring cellular proliferation.

FIGURE 1

Native sequence Chain 1	EICPAVKRDVDLFLTGTPDEYVEQVAQYKALPVVLENARILKNCVDAKMTEEDKENALSLLDKIYTSPLC
Peptide 1	EICPAVKRDVDLFLT
Peptide 2	RDVDLFLTGTPDEYVE
Peptide 3	GTPDEYVEQVAQYKAL
Peptide 4	EQVAQYKALPVVLENA
Peptide 5	YKALPVVLENARILK
Peptide 6	PVVLENARILKNCVDA
Peptide 7	RILKNCVDAKMTEEDKE
Peptide 8	KMTEEDKENALSLLDK
Peptide 9	NALSLLDKIYTSPLC

FIGURE 2


Native sequence
Chain 2
VKMAETCPIFYDVFFAVANGNELLLKLSLTKVNATEPERTAMKKIQDCYVENGLISRVLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR
Peptide 2.1
VKMAETCPIFYDVFFA
Peptide 2.2
IFYDVFFAVANGNE
Peptide 2.3
VANGNELLLKLSLTKV
Peptide 2.4
ELLLKLSLTKVNATEPE
Peptide 2.5
NATEPERTAMKKIQD
Peptide 2.6
RTAMKKIQDCYVENGL
Peptide 2.7
CYVENGLISRVLDGLV
Peptide 2.8
ISRVLDGLVMTTISSS
Peptide 2.9
MTTISSSKDCMGEAV
Peptide 2.10
KDCMGEAVQNTVEDLK
Peptide 2.11
QNTVEDLKLNTLGR

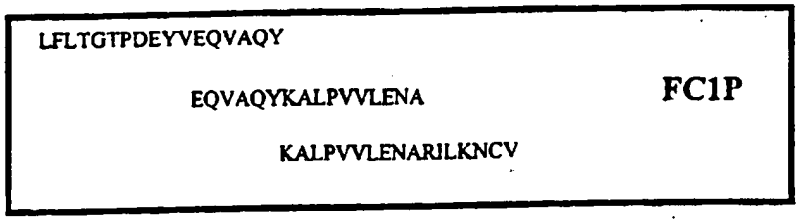

LFLTGTPDEYVEQVAQY
EQVAQYKALPVVLENA
FC1P
KALPVVLENARILKNCV

FIGURE 3

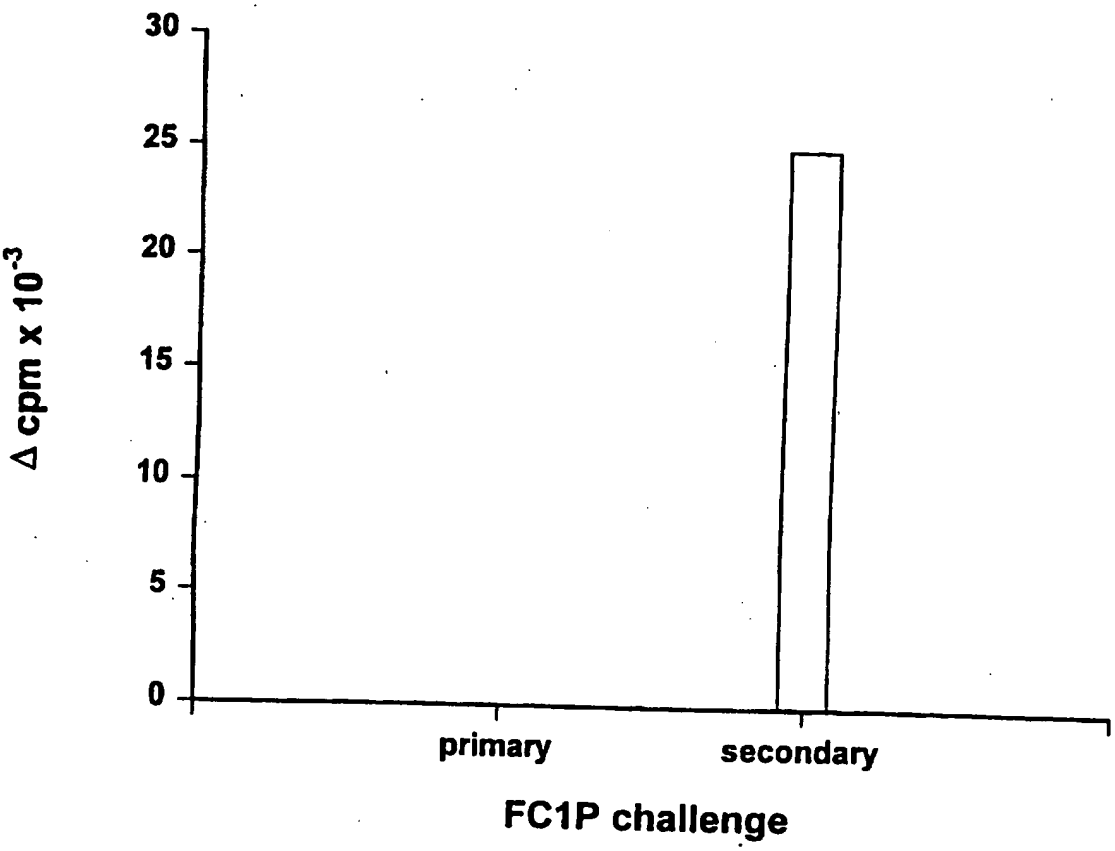


FIGURE 4

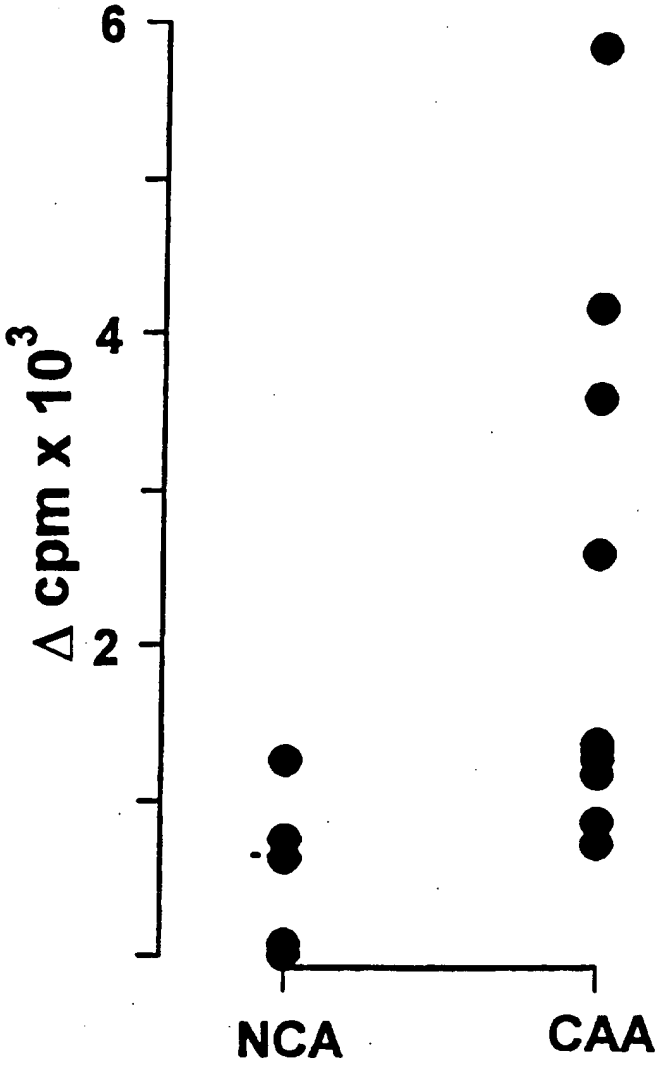


FIGURE 5

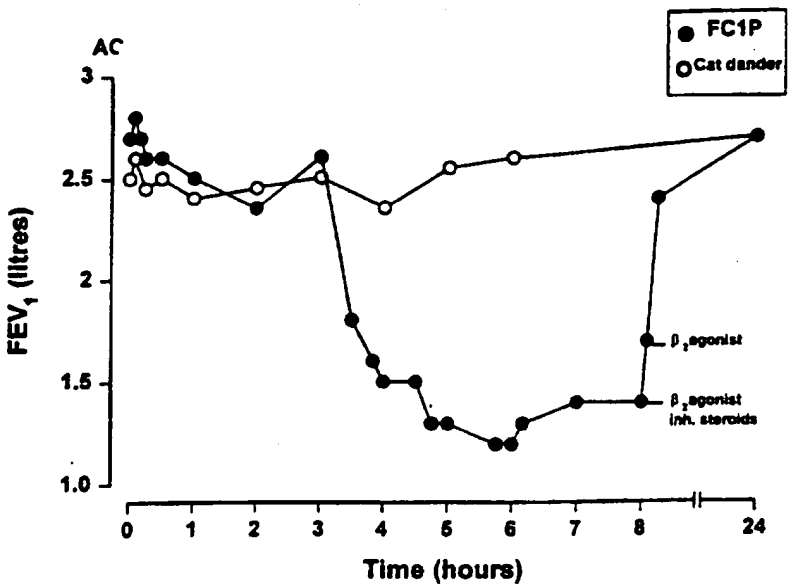
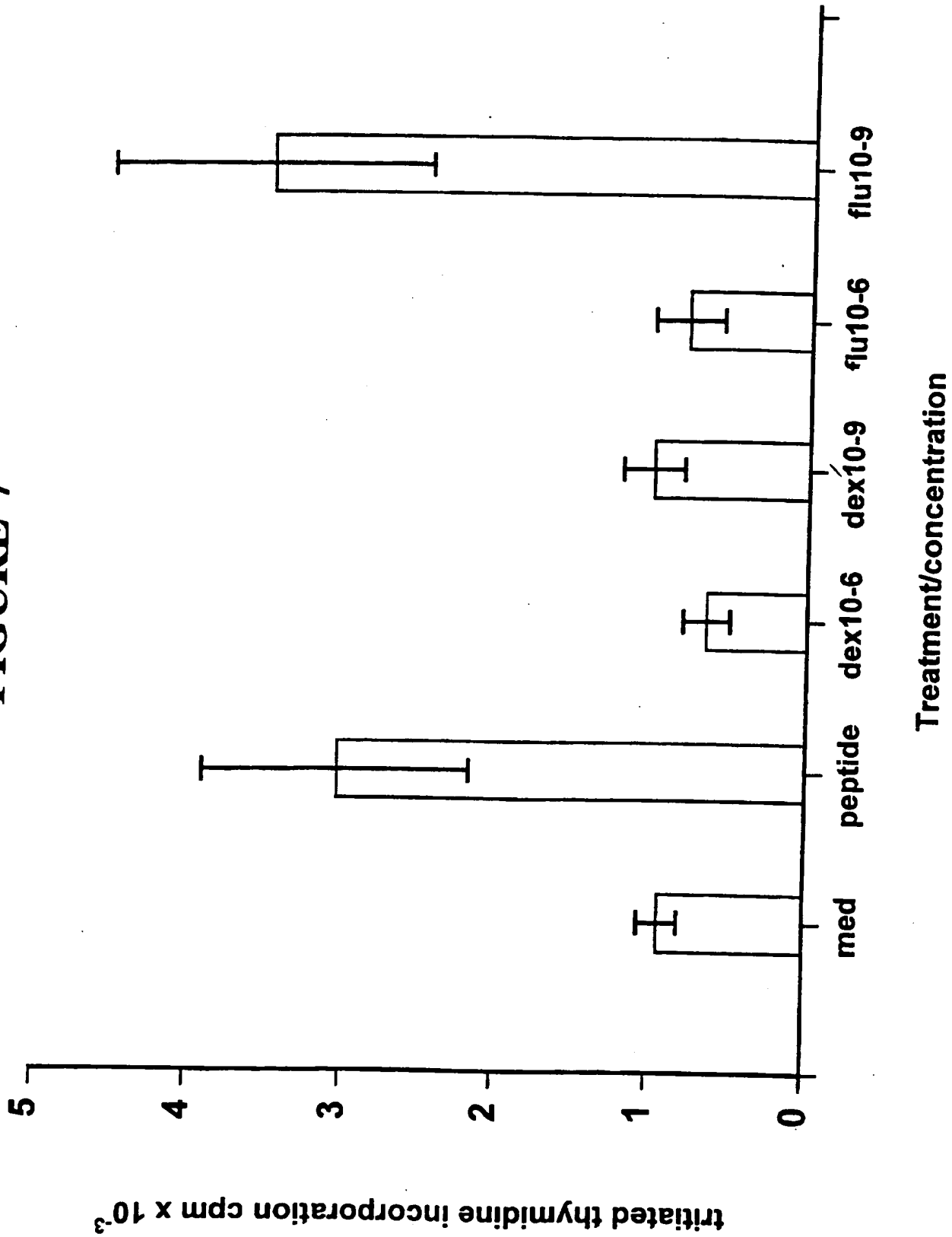

AC
FC1P
Cat dander
3
2.5
2
1.5
1.0
FEV1 (litres)
β2 agonist
β2 agonist
inh. steroids
0
1
2
3
4
5
6
7
8
24
Time (hours)

FIGURE 6

FIGURE 7


tritiated thymidine incorporation cpm x 10-3
0
1
2
3
4
5
med
peptide
dex10-6
dex10-9
flu10-6
flu10-9
Treatment/concentration

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/50 C07K14/435 A61K38/04 A61K39/35

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 08280 A (IMMULOGIC PHARMA CORP) 29 April 1993 see the whole document ---	12-19
X	WO 91 06571 A (IMMUNOLOGIC PHARMACEUTICAL CO.) 16 May 1991 see the whole document ---	12-19
X	WO 93 19178 A (IMMUNOLOGIC PHARMACEUTICAL COR) 30 September 1993 see the whole document ---	12-19
X	WO 96 07428 A (IMMULOGIC PHARMA CORP ;GEFTER MALCOLM L (US); SHAKED ZE EV (US); M) 14 March 1996 see page 9, line 27 - line 32 --- -/--	12-19

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
1 August 1997	20 -08-1997

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 27634 A (INST CHILD HEALTH RESEARCH) 8 December 1994 see page 4, line 2 - line 14 see page 10, line 27 - line 32; claim 10 see page 14 -----	1

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